



**UNIVERSITI PUTRA MALAYSIA**

**HUMAN HEPATITIS B VIRAL PROTEINS HBX AND HBE: ROLES IN  
HepG2 CELL LINE SURVIVAL AND CELL DEATH**

**ANDREA LISA HOLME**

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**HUMAN HEPATITIS B VIRAL PROTEINS HBX AND HBE:  
ROLES IN HepG2 CELL LINE SURVIVAL AND CELL DEATH**

**By**

**ANDREA LISA HOLME**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirements for the Degree  
of Doctor of Philosophy**

**January 2004**



## **DEDICATION**

**In Loving Memory**

**Of**

**Elizabeth Christie Holme**



Abstract of this thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirement for the degree of Doctor of Philosophy

**HUMAN HEPATITIS B VIRAL PROTEINS HBX AND HBE: ROLES IN  
CELL SURVIVAL AND CELL DEATH**

**By**

**Andrea Lisa Holme**

**January 2004**

**Chairman: Professor Datin Farida Jamal, M.B.B.S., M.Sc., M.R.C. Path.**

**Faculty: Medicine and Health Sciences**

Existing reports of viral hepatitis, resulting in liver cell death have not been fully explained with regards to the mechanism of the viral proteins involved. The objective of the study is to determine if any of the Hepatitis B viral proteins cause changes in the survival of human hepatocytes and if so by what means. The two main candidates for inducing survival changes were the precore proteins (HBE) and HBX, both of which have been reported to accumulate in the liver of patients and to trigger an immune response. The human liver HepG2 cell line was chosen to study the effect of these proteins during transient expression. The results from this study show that both viral proteins can induce cell death by an apoptotic mechanism via caspases. HBX appears to trigger more cell death than HBE, while HBE-induced an initial proliferation of the cell culture followed by cell death. HBX-induced apoptosis appears to involve both extrinsic and intrinsic cell death systems through the Fas

system and the mitochondria, respectively. There is also a total loss of the PI3K/Akt pathway survival signals. The HBE-induced apoptosis appears to be through DNA damage triggering an intrinsic cell death program, coupled with a partial loss of the PI3K/Akt pathway that allows GSK3 $\beta$  to be activated, while keeping FKHR inactive. In both cases, the viral cell death can be prevented using the correct dosage of IL-6 stimulation, while loss of serum or the addition of ethanol can have an overall positive effect on the viability of HBX and HBE transfected cells. The deaths can also be prevented in varying degrees by the inhibition of MEK1 and PP1A/2A suggesting these pathways are involved probably by cross talking.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai syarat memenuhi keperluan untuk Ijazah Doktor Falsafah

**PROTIN HBX DAN HBE VIRUS HEPATITIS B MANUSIA: PERANAN  
DALAM KEHIDUPAN SEL DAN KEMATIAN SEL**

**Oleh**

**Andrea Lisa Holme**

**Januari 2004**

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**Fakulti: Perubatan Dan Sains Kesihatan**

Laporan tentang virus hepatitis yang mengakibatkan kerosakan hati belum lagi dijelaskan dengan sepenuhnya dalam aspek mekanisma dan peranan protin virus yang terlibat. Projek ini bertujuan menyiasat sebarang protin virus hepatitis B yang mempengaruhi kehidupan sel hati manusia dan, jika ada bagaimana protin tersebut berfungsi. Dua protin yang memainkan peranan penting adalah protin precore (HBE) dan HBX. Kedua-dua protin tersebut telah dilaporkan terkumpul di dalam hati pesakit dan akan merangsangkan respon keimunan. Sel kanser hepatoblastoma, HepG2, telah dipilih untuk menyiasat kesan protin tersebut semasa transient ekspresi. Keputusan menunjukkan kedua-dua protin virus itu dapat merangsangkan kematian sel melalui mekanisma yang bergantung kepada caspase. HBX didapati merangsangkan kematian sel yang banyak berbanding dengan HBE. Manakala, HBE merangsangkan fasa awal pembahagian sel diikuti dengan kematian sel. Perangsangan apoptosis oleh HBX melibatkan sistem

kematian sel ekstrinsik dan intrinsik melalui sistem Fas dan mitokondria masing-masing. Terdapat juga kehilangan isyarat kehidupan bagi perjalanan PI3K/Akt. Kematian sel akibat daripada HBE adalah disebabkan oleh kerosakan DNA yang seterusnya merangsangkan program kematian sel intrinsik. Bersamaan kejadian tersebut, terdapat kehilangan separa dalam perjalanan PI3K/Akt yang membolehkan keaktifan GSK3 $\beta$  tanpa mengaktifkan FKHR. Kesan kematian sel akibat daripada kedua-dua protin virus ini dapat diterbalikkan dengan sukatan IL-6 tertentu. Manakala, kehilangan serum atau penambahan etanol boleh membawa kesan positif ke atas viabiliti sel yang dijangkiti HBX dan HBE. Darjah penyongsangan kematian sel dipengaruhi oleh penyahaktifan MEK 1 dan PP1A/2A. Kesimpulannya, kedua-dua protin virus tersebut berkerjasama merangsangkan kematian sel yang dapat dipengaruhi oleh factor-faktor luaran.

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I certify that an Examination Committee met on 5<sup>th</sup> January 2004 to conduct the final examination of Andrea Lisa Holme on her Doctor of Philosophy thesis entitled "Human Hepatitis B Viral Proteins HBX and HBE: A Role in Cell Survival and Cell Death" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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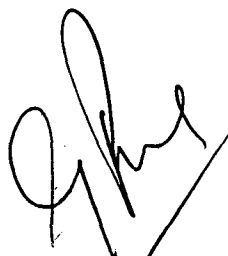
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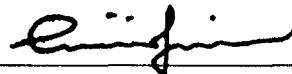
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**DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

**ANDREA LISA HOLME**

Date: 5<sup>th</sup> January 2004

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## LIST OF ABBREVIATIONS

<b>4E-BP</b>	eIF-4E binding protein
<b>Abl</b>	Ableson protein tyrosine kinase
<b>AKT</b>	Cellular homolog of the v-akt oncogene, an S/T protein kinase
<b>Apaf-1</b>	Apoptotic protease activating factor-1
<b>ASK</b>	Apoptosis signal-regulating kinase
<b>Bcl</b>	B cell leukemia oncogene
<b>Caspase</b>	Cysteine proteases with aspartate specificity
<b>CBP</b>	CREB binding protein
<b>CDK</b>	Cyclin-dependent kinase
<b>c-Raf</b>	Raf proto-oncogene S/T protein kinase
<b>CREB</b>	cAMP response element-binding protein, CREB1
<b>DAG</b>	Diacylglycerol
<b>DAPI</b>	4', 6-Diamidino-2-phenylindole
<b>DED</b>	Death Effector Domain
<b>DR</b>	Death receptor
<b>E2F</b>	Transcription factor family including E2F- and DP-like subunits
<b>eEF</b>	Eukaryotic elongation factor
<b>eIF</b>	Eukaryotic initiation factor
<b>ELK1</b>	Ets domain protein
<b>ERK</b>	Extracellular signal-regulated kinase, MAPK
<b>FADD</b>	Fas-associated protein with death domain
<b>FAK</b>	Focal adhesion kinase

<b>FasL</b>	Fas Ligand
<b>FasR</b>	Fas Receptor
<b>FKHR</b>	Forkhead in rhabdomyosarcoma
<b>FLIPs</b>	FLICE (Caspase 8) inhibitory protein
<b>GSK-3<math>\beta</math></b>	Glycogen synthase kinase-3 $\beta$
<b>HBE</b>	all precursor protein forms of Hepatitis B virus
<b>HBeAG</b>	secreted precursor protein
<b>HepG2-HBE</b>	HepG2 transfected cells with HBEpTARGET™ vector
<b>HepG2-HBX</b>	HepG2 transfected cells with HBXpTARGET™ vector
<b>IAP</b>	Inhibitor of apoptosis
<b>ICAD</b>	Inhibitor of caspase-activated deoxyribonuclease
<b>I<math>\kappa</math>B</b>	Inhibitor of NF- $\kappa$ B
<b>IKK</b>	I $\kappa$ B kinase
<b>INK4</b>	Inhibitor of CDK 4
<b>IRS</b>	Insulin receptor substrate
<b>ISRE</b>	Interferon-stimulating response element
<b>Jak</b>	Janus-family tyrosine kinase
<b>JNK</b>	Jun N-terminal kinase
<b>MAPK</b>	Mitogen-activated protein kinase
<b>MEK</b>	MAPK/ERK kinase, MAPKK
<b>MEKK</b>	MEK kinase
<b>MLK</b>	Mixed lineage kinase
<b>MTT</b>	Methylthiazoletetrazolium
<b>NF-<math>\kappa</math>B</b>	Nuclear factor kappa B



<b>NIK</b>	NF-kB Induced kinase
<b>NOS</b>	Nitric oxide Synthase
<b>p53</b>	Tumour suppressor protein that protects from DNA damage
<b>PKD</b>	3-phosphoinositide-dependent protein kinase
<b>PH</b>	Pleckstin homology domain
<b>PI3K</b>	Phosphoinositide-3 kinase
<b>PIAS</b>	Protein inhibitors of activated STATs
<b>PKA</b>	Protein kinase A
<b>PKC</b>	Protein kinase C
<b>PKR</b>	dsRNA-dependent serine/threonine protein kinase
<b>PP1</b>	Phosphoprotein phosphatase 1
<b>PP2A</b>	Phosphoprotein phosphatase 2A
<b>PP2B</b>	Phosphoprotein phosphatase 2B
<b>PYK2</b>	Proline-rich tyrosine kinase-2
<b>PCR</b>	Polymerase Chain Reaction
<b>RAIDD</b>	RIP-associated ICH/CED-3-homologous protein with a death domain
<b>RIP</b>	Receptor-interacting protein
<b>SAPK</b>	Stress-activated protein kinase
<b>Shc</b>	SH2-containing collagen-related proteins
<b>Smad</b>	Contraction of Sma and Mad (Mothers against decapentaplegic)
<b>TEN</b>	Phosphatase and tensin homolog deleted on chromosome ten
<b>TNF</b>	Tumor necrosis factor
<b>TRADD</b>	TNF receptor-1-associated death domain protein